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Synthetic Biology: Designing Novel Organisms for Biotechnological Applications

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Annotation: Synthetic biology is an emerging field that aims to design and genetically engineer novel organisms and biological systems for biotechnological applications. Rapid advancements in the field may yield disruptions in several industries, all the way from sugar and crude oil production to drug discovery and biomedicine. Synthesis of novel bioactive natural products may become cheaper than isolation from native sources, while bioremediation may revolutionize the oil, metal, and plastics industry. Synthetic biology encompasses several innovative engineering concepts for rationally designing biological systems, which are mathematically predictable. The design scope ranges from small, engineered proteins to large, transgenic organisms, or even assemblies of such organisms. At this moment, the full scope of possibilities is largely unexplored. It is thereby imperative for natural scientists to investigate and understand the myriad of possible implications of this rapidly expanding field—both in terms of basic science and public concerns. One of the main applications of synthetic biology is the transformation of microorganisms into green factories for the high-yield production of biofuels, pharmaceutical precursors, non-protein amino acids, and industrially challenging enzymes. Owing to the various applications, the field is heavily funded, with investment figures in the billions of US dollars.

Although a rapidly advancing field, there are major challenges in synthetic biology, such as the difficulty in inserting large engineered pathways into genomes while accomplishing near-native expression and without disturbing the host's native metabolism. However, the effects of design interventions are starting to gain predictive power, setting the stage for improved genetic circuitry. In order to maximize potential social benefit and minimize the drawbacks, it is important that ethical considerations keep pace with developments.

1. Introduction to Synthetic Biology

Synthetic biology comprises various fields, methods, and research practices that share a few common foundational characteristics. Before one can further discuss the scope of synthetic biology, it is necessary to clarify these basics. Synthetic biology is typically proposed to represent a new technological design paradigm. It is supposed to counter the limitations of design in the life science fields. Therefore, it is equally important to clarify that, unlike countless genetic modifications, generated organisms and biological systems are not unnatural. They still consist of biological components and obey the laws of physics and chemistry, and all current knowledge about biology, and are thus different from the new biology of artificial living systems. A bottom-up definition will be provided in the ontology to illustrate its relationship to molecular systems biology within the design lifecycle of biological components and devices. Synthetic biology can therefore be understood to describe the (re-)engineering of existing naturally evolved systems rather than the creation of fundamentally new biological systems. The visions and approaches considered refer to the development of well-understood and standardized biological components that are hierarchically structured and can easily be assembled to form more complex biological systems.

The most tangible consequence of definitional clarification is a reorientation of the principal discussion. The newly defined synthetic biology can be seen as a key driver in an ongoing interdisciplinary approach based upon a new paradigm of the engineering of biological systems. The life sciences will integrate physical and chemical methods with advanced biological knowledge to build new biotechnologies that could justifiably be framed as complementary. A new integrated theoretical approach is called for, which conceptually describes the state of the art and allows the identification of deficits to set priorities for research and development. Discussion of potential and limitations should be based on applications in order to be fruitful. Synthetic biology may contribute to providing solutions to so-called grand challenges, provided a constructive dialogue with society takes place. [1][2][3]

1.1. Definition and Scope

Synthetic biology can be defined as the design and construction of novel, artificial biological parts, devices, and systems that do not exist in nature, and as the redesign and fabrication of genetic circuits which give living organisms novel functions. Synthetic biology is often seen as an interdisciplinary field that combines aspects of biology, engineering, and computer science. Indeed, synthetic biology can be seen as a biological engineering discipline. The scope of work in synthetic biology is somewhat loosely defined, but is generally agreed to be an area of research and development that focuses on the redesigning of biological systems and organisms to make them more useful to humans. This could include constructing synthetic cell-like units with capabilities that do not exist naturally, new metabolic functions, the ability to bind and treat human disease, sensors of chemical agents and pathogens, etc. In addition to cells and cell-like systems, it could include new forms of plants and animals. The development of new organisms is a core part of this strategy, as bioproduction in these industries can benefit most from microbes, cells, and other organisms that have been designed and constructed for a specific human purpose. Synthetic biology research could therefore lead to applications in several different industrial areas, agriculture, chemicals, pharmaceuticals, food, etc. The development of new organisms can consist of computational simulations, cellular modeling, standardization of components, and the local/global construction of artificial cells. Synthetic biology is not about new processes for GM technology alone. Synthetic biology is not a current 'big science.' Synthetic biology is not 'systems biology'—the latter uses existing biological systems to gain insights into cellular mechanisms and understand and predict biological functions. Synthetic biology can include some elements from systems biology research or help systems biologists test certain hypotheses. Some systems biology experts also emphasize that constructing new cells is not a goal of their research. A number of ethical, social, economic, legislative, and intellectual property rights issues are associated with synthetic biology approaches. It is not possible to discuss these here. In addition, none of the issues is specific to synthetic biology and would apply to genetic engineering equally. [4][5][6]

1.2. Historical Development

The concept of synthetic biology has existed for many decades. However, only in the last 15 years has the field become recognized as a distinct discipline. Attempts to modify bacteria to produce novel outputs have a long history in genetic engineering and biotechnology; one often-cited example is that of the first splicing of genes in 1973. In molecular biology, numerous 'firsts' have taken place, each of which became significant as researchers further built on initial successes. For instance, in 1951, an influential experiment in isolation of a new strain of Escherichia coli led to the development of techniques of replica plating and bacterial mutation. Following this, genetic investigations using these techniques led to increasingly refined and detailed descriptions of how natural living organisms function. Over the years, genetic engineering research began to focus on manipulating an organism's DNA to introduce new or improved functionalities. The study of systems biology, which considers both the genotype and phenotype as interacting systems, is a more recent development.

Synthetic biology has been described as 'the application of engineering principles to the design of biological systems'; yet, decades earlier, this philosophy was encapsulated in a book that reiterated the belief of many systems theorists that 'construction, not analysis, is the only way to prove that we understand a system.' Although the term 'engineering' was not explicitly used, it was described how scientific control and industrial exploitation might overcome the constraints of natural variation. Indeed, the search for improved production organisms in modern biotechnology has presented new opportunities. Furthermore, just as genetic engineering emerged as a distinct scientific and industrial discipline in the wake of recombinant DNA techniques, the development of DNA synthesis and assembly technologies, along with genome sequencing and 'high-throughput' methods for biological discovery, have more recently led to the identification of new challenges and opportunities. [7][8][2]

2. Fundamental Concepts in Synthetic Biology

Fundamental concepts in synthetic biology are necessary background for any deeper understanding of the techniques and approaches making up this interdisciplinary research field. Synthetic biology commonly deals with the design and construction of genetic circuits in novel organisms for biotechnological applications or with the modification of existing organisms in some way. Genetic circuits are composed of biological parts, including genes, promoters that initiate DNA transcription, regulators, and the sequences they bind to, and the genes that respond to regulation. Parts are used to construct a genetic circuit, making possible the transformation of one or more inputs into one or more outputs that perform some desired function. In the early days of synthetic biology, biological parts were used mostly in isolation, as opposed to being thought of as a modular system or system of systems. The task for the synthetic biologists has been to identify parts or to design them such that they can perform predictable functions and to figure out how to put a number of such parts together to produce a circuit with a required function. Moreover, the idea of standardization of parts, both in design and behavior in different circuits, has been a major innovation in the subject.

In sum, a genetic circuit consists of a number of parts, the function of which is to regulate the product of one or more genes. Changes in the regulation and/or the gene circuit itself will create different regulatory behaviors, leading to a new output. One of the central problems in designing a synthetic biological system involves how to make sure that the intended parts can be identified and interact in the desired way, if and when they are combined. Necessary technological innovations have enabled the experimental and computational manipulation of complex biology over the past several years. In the experimental realm, precision in genetic manipulation and growth control of microbial systems has advanced the ability to design and create synthetic gene networks, modules, pathways, and even entire genomes. Moreover, combinational approaches that integrate a number of mutations or transmutations are difficult to predict unless they have been pre-existing in natural organisms, because of the enormous number of combinations to be considered when multiple mutations are involved. The efficient construction of these kinds of biological systems will be possible only if the engineering promise of standard parts is realized. This will involve rethinking and revising much of the current methodology employed by synthetic biologists. At the same time, many of the domestication functions have parasites in their neighborhoods; elimination of these elements may be more critical than the transformation of the native regulatory networks themselves. Although making sure that all the basic mechanisms are designed and experimentally functional, how would one be able to ensure that when they are put together, they will simply follow instructions? This is still a major challenge despite the advent of standardization and modularization innovations. [9][10][11]

2.1. Genetic Circuits and Modules

Among the most fascinating developments in synthetic biology has been the design, and increasingly, the implementation of genetic systems that operate as circuits and modules with specific functions. Genetic circuits operate by directing the expression of genes and affecting other properties of a cell, much as electronic circuits direct current flows in various devices. As in electronic systems, a variety of functional components can be combined to produce a broad array of phenotypes. The components used in genetic circuits are termed genetic modules and include sensors (to detect intracellular or extracellular signals), switches (to change the behavior of the circuit in response to signals), oscillators (to produce the type of time-dependent behavior seen in a variety of dynamic phenomena), and actuators (to produce useful work, such as manufacturing a biotech product, including the components of other genetic circuits).

Genetic modules, in turn, are built from other genetic modules and their underlying biological components. For instance, it is necessary and often desirable to link a biological sensor to a switch via an appropriate data integration process. All of these modules, some gleefully and others unexpectedly, have been implemented in a number of host organisms, illustrating the extensive degree of control over the flow of genetic and related information originating in a gene that we now possess. We need to be careful with our control, of course, and that is one of the themes of this paper. Investigators have also sought to interlink modules so that the output of each module serves as a signal governing the operation and output of other modules. This allows the construction of more complex systems, somewhat like building a car from sub-assemblies. This paper is about the design and engineering of the basic modules from which to assemble new or refined genetic systems on our pathway to novel organisms and increased public wellness. [12][13][14]

2.2. Standardization and Modularization

Standardization involves making the smallest divisible units of a device or a system compatible and interchangeable with all others, thus enabling more efficient construction of the final device or system. Analogous to current genetic bioengineering, this has led to the distinction of an entire scientific field within biological synthetic biology called standards or physical bioinformatics. Standardization has allowed advanced biological engineers to repeatedly construct complex chimeric molecular systems by simply assembling them from a stock library of standardized and modular components with predefined behaviors. The advantages of standardization and modularization in synthetic biology, i.e., that the smallest biological part (module) can be designed and whose desired function is constructed and readily combined with other parts to create, on the fly, the most unique and unprecedented biological circuit, pathway, or metabolic system are genuinely desired. Research is ongoing to develop algorithms and tools for building and verifying synthetic genetic networks in cells as well as to generate large collections of designed genetic promoters for plant synthetic biology.

The advantages of modularization for both genetic and non-genetic benchmarks are great, and ongoing standards development and research will greatly expand the scope and power of current and future synthetic biology design practices. However, current investigations in synthetic biology tell us that the engineering of synthetic organisms is complex and much more difficult than many engineers would believe. The design of bacterial systems is extremely predictable. Given the genetic circuits and regulatory modules that are presently understood, much of learning how to create and establish new genetic regulatory networks in bacteria, in the near term, is trial and error. [15][16] [15][16][17]

3. Tools and Techniques in Synthetic Biology

Dr. Technology Development: Tools and Techniques in Synthetic Biology Synthetic biology intersects heavily with DNA sequence-level research because it aims to design and construct new organisms. Naturally, over the past few decades, an assortment of technologies has been developed to facilitate these goals. Breakthroughs in DNA synthesis and assembly have enabled flexible creation of genetic sequences for broad purposes. In particular, new methods for primerless DNA assembly have been used to create novel protein domains from proteins, which in turn enabled precise high-throughput functional genomics assessing gene essentiality. Additionally, decreasing DNA synthesis prices have given rise to de novo synthesis of entire organismal genomes. In 2010, a team of researchers synthesized the 1.08 Mbp genome of a bacterium from scratch. The synthetic genome was inserted into the hollowed receiver cell and took over the cellular machinery.

As DNA synthesis becomes cost-effective, the field has moved towards embracing high-throughput, automated workflows and miniaturized reaction volumes. These efforts, in many cases, have culminated in the development of commercial synthesis and assembly platforms ready for widespread industrial use. The leap from traditional molecular biology to synthetic biology was facilitated by the development of robust, efficient, and precise methods for altering complex, multicellular genomes. Traditional genetic engineering is built around homologous recombination, a naturally occurring genetic recombination process. Homologous recombination enzymes, such as restriction enzymes, which cut DNA at specific locations recognized by a standard, double-stranded DNA sequence, have advanced significantly over the years. Able to work at high precision and on a large throughput, they revolutionized recombinant DNA technology decades ago and remain a standard for most applications. The discovery and worldwide emergence of the RNA-programmable, protein-endonuclease system was transformative for synthetic biology. With the development of CRISPR-Cas9, the biological manipulation of any organism, including mammals and humans, became more accessible and precise than ever before. [18]

3.1. DNA Synthesis and Assembly Methods

Synthesis and Assembly

3.1. DNA Synthesis and Assembly Methods DNA synthesis and assembly methods have evolved from traditional cloning to be a rapidly expanding field fundamental to synthetic biology. The development of high-throughput DNA synthesis platforms has reduced the cost of base-pair assembly efficacy significantly. Furthermore, these platforms are highly scalable, with synthesis pools capable of producing hundreds of thousands of oligos, resulting in the generation of exabytes of sequence data. Polymerase chain reaction (PCR) is perhaps the most widely known and used method of amplifying specific DNA sequences. It utilizes temperature cycling to change the physical state of synthetic DNA fragments through repeated rounds of thermal denaturation and polymerase extension. The resulting DNA fragments can then be propagated further for study or other molecular techniques.

The Gibson assembly is also a common method of assembling genes or other DNA elements such as promoters, terminators, or polyadenylation signals that have been PCR amplified or synthetically produced in vitro. Gibson assembly uses the 3′-5′ exonuclease activity of T5 DNA polymerase to degrade primers annealed to the DNA fragments, leaving single-stranded regions of DNA complementary to other fragments. Such single-stranded DNA is then annealed to neighboring DNA during extended incubation with Taq DNA ligase, resulting in the formation of covalently closed DNA without the need for restriction enzyme-based cloning. PCR and Gibson assembly exemplify the range of methods for the physical assembly of genetic constructs. Each of these has applications in gene therapy, genome editing, synthetic biology, and other genetic engineering domains. Host organisms have evolved complex DNA repair systems which make rapidly assembling long DNA sequences with a low error rate a significant challenge. [19][20]

3.2. Genome Editing Technologies

Genome editing

Technical revolutions have transformed synthetic biology in recent years. Especially genome editing technologies are revolutionizing our capabilities to modify or rewrite genetic material or even to write it from scratch in a defined way. The technologies are extremely versatile and are easy to implement. The basis of CRISPR technology is a sequence-specific nuclease, combined with a short-guide RNA. The gRNA sequence hybridizes with a complementary target sequence in the DNA, which can even become present in a plasmid. This causes a double-stranded break, which is then either repaired through organisms' endogenous repair machinery or by homology-directed recombination using an exogenous repair template.

This and other genome editing technologies have found numerous applications in different fields: in agriculture, they have been used to develop new plant traits or even crops, or in animal production for improved immune response. In medicine, genome editing holds the prospect of curing genetic diseases by correcting disease-causing mutations, bio-crops for pharmaceutical production, and many others. Genome editing also has the potential to create new enzymes or metabolic pathways for applications in environmental biotechnology. Nevertheless, genome editing has raised some ethical and safety concerns. Especially the increasing number of do-it-yourself biologists who arm themselves with kits fear challenges yet to be met, such as off-target effects, i.e., the possibility to modify unpredicted DNA sequences besides the intended ones, as well as the development of regulatory frameworks to ensure its responsible application. [21][22]

4. Applications of Synthetic Biology

To illustrate the power of synthetic biology to create new designs for novel organisms for a wide range of applications, let us solely consider its possibilities. Synthetic biology has the potential to revolutionize industrial biotechnology by providing the possibility to improve any biological organism for the widespread biosynthesis of natural or man-made products. Synthetic biology techniques could be used to optimize industrial microbial strains with an enhanced capacity to make fermentation products like alcohol or products derived from fermentation like vinegar, fructose syrup, and amino acids. Indeed, many bacterial and yeast strains have been biotechnologically improved in this way. Industrial biotechnology is a fast-growing field with great potential. Consumers in the developed world are increasing demand for sustainable alternatives to traditional, petroleum-derived chemicals and materials. Using synthetic biology might allow cells to make a vast array of new bio-products with new properties from renewable and alternative resources. Bacterial hosts have recently been engineered to produce bioplastics, biofuels, and green chemicals for use as solvents and precursors to complex medical molecules.

The most compelling application of synthetic biology to acknowledge is engineering biological systems to improve human health. A cell is a molecular factory and, because the production and/or associated properties of these factories can be altered using the tools of synthetic biology, the capabilities of a cell can be harnessed to address challenges in biotechnology, health research, and medicine. In medicine, synthetic biology offers possibilities in two main areas: diagnostics and therapeutics. Cells could be made to use as drug delivery devices or biosensing devices. The immune response of doctors and patients to therapies and vaccines could be better tested and predicted in the laboratory using a model synthetic system than using laboratory animals. We could also reduce the amount we need to rely on rare human or laboratory animal infected tissue samples. There are also many other capabilities that this technology opens up. Agricultural science could be enhanced to produce crops that make high-value pharmaceuticals or characteristics otherwise difficult to obtain. Synthetic biology could be an effective aid in environmental remediation. For example, pollutants such as mercury or other hydrophobic organics could be sequestered to a non-toxic state by modifying the metabolism of plants or bacteria. The need for non-toxic methods for decontamination from radioactive materials makes this area of multidisciplinary interest. [5][23]

4.1. Industrial Biotechnology

A key application of synthetic biology is in the area of industrial biotechnology. In particular, industrial biotechnology has been a driving force for numerous companies in the area of ingredients and specialty chemicals, for liquid and gas fuels, as well as for materials. For many of these applications, the ultimate goal is to engineer microorganisms that can sustainably produce desired end products, replacing what is done today with chemical refineries, the extractive oil industry, or even daily farming. Replacements come in all shapes and forms, from small chemicals, such as solvents and flavor compounds, to much bigger monomers, like alkanes or alkenes used to produce polymers, to end products such as some natural additives and sweeteners. Currently, many manufacturers and venture investors in this space are looking for 'smarter' ways to make 'things,' investing in facilities and processes that are more resource-efficient and generate less waste. At the heart of what investors are looking for is 'bioprocess optimization,' or how to do these fundamentally more sustainable processes even better in a transformed planet. All of these initial applications involve some engineering, some amount of better bioprocess optimization, and some addressing of cellulose conversion and cellulosic feedstocks for biotechnology that are commodities. Besides the need for engineering organisms, there is often a huge requirement for harnessing better process-based, and even fundamentally alternative, biochemical reactions in an organism with the goal of more sustainable productivity. There are several case studies in industrial biotechnology where companies have successfully developed engineered organisms for industrial applications. While there are many motivations for this field of application, this is indeed a field where investment and funding are already starting to apply the vision of ostracizing GHG-producing or GHG-using underpinning industries and producing an alternative end-goal approach. Of course, this presents hurdles to such advocates, such as scaling or market acceptance, legislators' will, investment, and competition. Industry and scientists in this area for these many applications need to work in very close-knit partnerships to determine possible applications and for engineered functionality to move to actual products. [24][25]

4.2. Medical Applications

The power of synthetic biology to design and engineer novel organisms for a specific purpose has the potential to make huge changes to healthcare. This includes the production of new drugs, designing single-shot curative therapeutics to reset genetic errors, new vaccine development, and the use of engineered organisms for diagnosis and treatment for conditions that need a patient-specific diagnosis – personalized medicine. Key features, such as the ease and speed of genetic engineering enabled by synthetic biology, that have the potential to transform these areas are highlighted. Ethical, legal, and social implications of the medical potential of synthetic biology are also introduced, with a larger section dedicated to discussing these issues. Do biological systems have the potential to be used in a radically new way in healthcare? Yes – because they can replicate and evolve in response to a changing diagnostic and treatment environment in real time. The ability to genetically program and reprogram organisms to perform new tasks can be seen clearly in some of the areas of new product development and delivery that are using biology as synthesized by real natural or non-genetically modified cells or microbes to deliver the active ingredients for a one-shot cure in a new and more efficient way. In the area of producing high-value drugs, cells with different circuits that make them act as production factories can be injected directly into the patient's body. Efficacy trials in models are advancing. Antigen chimeras with T cell receptor cells are isolated from the desired patient, and a set of viral vectors are used to express in the cells a bespoke cocktail of synthetic protein-producing gene add-ons. These synthetic proteins allow the cell to recognize and bind to the required unique molecular feature found on the surface of the patient's tumor, generating a response. [16][26]

5. Ethical and Regulatory Considerations

The increasing interest in creating novel organisms for biotechnological applications has raised concerns about the safety of 'engineering' life. These concerns are reinforced by the dazzling advances in systems and synthetic biology – areas thought to rejuvenate industrial biotechnology. In an attempt to equate ethical concerns with environmental hazards and risks of bioterrorism, synthetic biology has been constructed as a field in which engineering would be applied to biology. The safety of synthetic biology is often considered under the rubric of 'dual-use' and 'responsible research and innovation.' However, the focus on regulation-worthy hazards that would follow from the misuse of synthetic organisms is too narrow.

In transnational conversations we have conducted on the ethics of synthetic biology, integrated into the public engagement activities of the project, several concerns have been raised. Are we paying sufficient attention to stewardship and care in altering living beings? What does it mean to create a novel form of life and unleash it in an ecosystem? Are we already overpopulating the world with artificial forms of life we may not be able to control? Do we need to consider the ethics of stopping synthetic biology as seriously as the biosecurity implications? While it is difficult to find general answers to these kinds of ethical questions, what is clear is that the development and potential benefits of synthetic biology should not take place at any cost. Together with new innovations, solid regulatory mechanisms are needed to prevent misuse, as well as to limit and control the environmental impact and interactions of novel organisms. Synthetic biology's novel pathway of integrating rational design and large-scale DNA assembly may create new safety and biosecurity challenges. In this context, responsible innovation and the proactive involvement of society in synthetic biology developments, opportunities, and likely impacts are crucial to foster more mature innovation processes and bring about technologies that are ethically acceptable and finally sustainable. In order to build and support broad and inclusive dialogues, there is a need to evolve viable ways to ethically and effectively engage the public and other relevant stakeholders in these complex issues.

6. Conclusion

Advances in the fields of genetics, biochemistry, and cell biology over the last half-century have provided the tools needed to consider revisiting 'canned' biotechnology, constructing new organisms and biotechnology from the bottom up for the multitude of novel functions of our choice. This new field of research, synthetic biology, is now a growing and dynamic area of research, with many potential applications that could help solve some of the world's most pressing 21st-century challenges. The breadth and depth of tools developed, or currently under development, to explore, manipulate, and exploit the natural world have grown at a similar pace to the extent that as a global society we now have the power, if not as yet the wisdom, to influence the genetic makeup and functions of our planetary life forms and ecosystems. To ensure that the technological means of constructing and using novel organisms will be beneficial to humanity, society, and the environment, they must be developed and regulated based on our best understanding of the science as well as the potential social and cultural impacts of synthetic biology. Thus, interdisciplinary work on the potentials and impacts of synthetic biology spans a wide range of research interests, from the laboratory bench to the corridors of power.

Engineering novel organisms can already be conducted at laboratory scales, and work is in progress to design and couple bespoke organisms into systems that are able to replicate and evolve novel functions and adapt to environmental pressures. New innovative energy sources, medicines, foodstuffs, biosensors, and decontamination are but a few of the many biotechnological applications that are potentially enabled by such technologies. Furthermore, applications in areas such as homeland security, military installations, and environmental management are also envisaged, steered by a cornucopia of organisms with tailored and discrete functions designed for action in the field. However, even a technology that has not fully matured can engender debate about responsible stewardship by its practitioners. Synthetic biology has the potential to revolutionize many industries, but concerns have been raised that life invented and designed by humans operates in a risky and potentially unpredictable area, a point that is very difficult to argue against. Ongoing laws and regulations are, therefore, currently the main means through which work in this area can navigate between responsible stewardship and unlocking the full potential of biology.

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